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RESEARCH ARTICLE

Improvement of Microbial Fuel Cell Electricity Generating with Bacterial Isolate Implementation on Electrode in Wastewater of Pindang Fish Processing

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Abstract

Microbial Fuel Cells (MFCs) generate eco-friendly electricity from organic matter through microbial activity without producing CO_2 emissions. This study examined the effects of bacterial adhesion (Pediococcus acidilactici and Staphylococcus warneri) and activated sludge addition on improving MFC electricity generation, reducing pollutants from fish processing wastewater, and increasing microbial density on electrodes. The study comprised five stages: bacterial cultivation, bacterial adhesion on electrode surfaces, activated sludge acclimatization, MFC assembly, and electrical measurement. Four treatments were applied: electrodes without bacterial attachment and without activated sludge (Ko), electrodes without bacterial attachment but with activated sludge (KoL), electrodes with bacterial isolates attached (anode with Pediococcus acidilactici and cathode with Staphylococcus warneri) without activated sludge (AK), and electrodes with bacterial isolates attached with activated sludge (AKL). The AKL treatment achieved the highest average electrical output, with a voltage of 0.31 ± 0.02 V, a current of 3.28 ± 0.66 mA, and a power of 1.15 ± 0.25 mW. Pollutant loads, measured by COD, BOD, and TAN parameters, decreased by 57%, 38%, and 92%, respectively. Bacterial adhesion on electrode surfaces combined with activated sludge addition significantly increased bacterial density on the electrodes. These results indicate that microbial interactions and activated sludge enrichment enhance both electricity generation and wastewater treatment efficiency, highlighting the potential of MFC technology as an eco-friendly solution for sustainable energy production and waste management.

Keywords: bacterial fixation, bioelectricity, microbial fuel cell, *Pediococcus acidilactici*, *Staphylococcus warneri*.

Introduction

Pindang is the Indonesia preserved fish product that is processed by combining boiling and salting. The processing of pindang fish not only produces a product but also a byproduct in the form of waste, especially liquid waste resulting from the washing process and boiling fish. Liquid waste contains a large amount of fish protein and fat, which potentially increases the BOD5 and TSS content. Environmental pollution commonly occurs due to wastewater processing, resulting in an unpleasant odor. Pindang fish wastewater can be utilized in various ways; even wastewater from salt-boiled fish processing can be used as electrical energy generated with microbial fuel cells (MFC). This technology can generate electricity from organic substances by anaerobically oxidizing them with the help of bacteria. Exoelectrogenic bacteria can help in the process of electrical formation in MFC because the bacteria can help in the transfer of electrons directly in the process of electrical formation in MFC [1].

Bacteria are an important factor in MFC because they produce electrons. Electricity generated by MFC can be formed owing to a potential difference between the two electrodes. Microorganisms in MFC form aggregates or biofilms on electrodes. Without bacteria, MFC produced 0.18 V, while adding 2 ml bacteria increased it to 0.23 V [2]. Biofilm density positively correlated with electricity output from fish wastewater. Twelve bacterial isolates from the cathode showed diverse gram reactions, shapes, motility, and enzymatic activities [3].

Sequencing results from the 16S rRNA analyzer show that the biofilm on the anode surface consists of a blend of different species of microbes instead of single microbial species. The predominant phyla are Alpha Proteobacteria, Fermicutes, Gamma Proteobacteria, and Actinobacteria [4]. One variant of bacteria from the fermicutes family was found in electrode biofilms, namely *Pediococcus acidilactici* DSM 20284 and *Staphylococcus warneri* NCTC 11044 [5]. *P acidilactici* dan *S warneri* are indigenous bacteria isolated from biofilm that form on electrodes.

Therefore, this study was conducted to determine the effect of bacterial isolates on the resulting MFC electricity, changes in the load of pollutants produced by wastewater from boiled fish, effect of bacterial paste on electrode plates, and the effect of adding active sludge to the density of microbes on the electrode plate.

Materials and methods

Materials

The material used in this study was fish processing wastewater from the Cindy Group Company in Parung, Bogor. Other materials used are bacterial isolates from activated sludge, Na-Alginat (Setia Guna), nutrient agar (NA) (Merck-105450), nutrient brain heart infusion (NBHI) (Merck-110943), thioglycolate broth (TB) (Merck-108190), bacto agar (Merck-100417), H_2SO_4 (Merck-160313), citric alkaline solution (Merck-100243), sodium hypochlorite (Merck-105614), phenol solution (Merck-840015), sodium nitroprusside (Merck-567538), 70% alcohol, and aquadest.

Tools used in this research are jerry cans, one vessel MFC tool with dimensions of 10x7x10 cm3, glass, graphite rods, copper electrodes, cables, dynamos, sudip, test tube (Pyrex), screw reaction tube (Pyrex), petri dish (Pyrex), anaerobic jar, volumentric pipette (Pyrex), tristation device (Pyrex), winkler bottle (Durrant), cup glass (Pyrex), buret (Pyrex), DO meter (Lutron DO-5510), UV-VIS spectrophotometer (Optima SP 300), pH meter (TOA HM 30V) , and the Osmond DT-9205A multimeter).

Methods

This research was conducted with five stages, namely the main stage of bacterial cultivation, the second stage of bacterial isolates in electrodes, the third stage of activated sludge acclimatization, the fourth stage of MFC design making one vessel, and the last stage of measurement of MFC electrification.

Pediococcus acidilactici and *Staphylococcus warneri* bacteria derived from activated sludge are incubated at 37^oC for 24 to 48 hours. The incubated culture was then inoculated at 10 mL media nutrient brain heart infusion (NBHI) and incubated for 24 hours at a temperature of 37^oC.

Acclimatization was performed by mixing activated sludge with fish wastewater at a volume ratio of 1:3. Mixing was carried out for 48 h with aeration on a mixture of active sludge and fishspawning waste. Fish production waste was subjected to pollutant load tests before mixing, which included the analysis of chemical oxygen demand (COD), biological oxygen demand (BOD), total ammonia nitrogen (TAN), and pH.

COD analysis can be performed by diluting the sample using an aquades up to 25 times dilution. The tool used was a closed reflux tube washed with H_2SO_4 20%. The sample (2.5 mL) was inserted into a reflux tube, and 1.5 mL of the digestive solution and 3.5 mL of sulfuric acid solution were added. The standard solution was prepared by mixing the detractor solution with a sulfuric acid solution (1.5 mL and 3.5 mL, respectively). The standard solution was then added to the reflux tube. The reflux tube was then closed and homogenized using a vortex. Reflux tubes were placed on COD reactors that had been heated to 150 °C for 2 h. The hot samples were then cooled and measured using a UV-VIS spectrophotometer at a wavelength of 600 nm. BOD value analysis can be done with a sample of waste diluted with a dilution factor of 10 times with a solution volume of up to 200 mL using aquadest in a closed erlenmeyer tube, then aerated sample for 30 minutes. The sample was divided into two bottles for incubation and DO measurement. The first bottle for incubation uses a special BOD bottle. The sample is incubated for 5 days in a dark place with a temperature of 20^{o}C. Tan value analysis was performed by inserting a 25 mL sample into the Erlenmeyer flask. Samples were added to phenol solution (1 mL) and homogenized. The sample was re-added to a 1 mL nitroprusside solution and homogenized. An oxidizing solution (2.5 mL) was added to the sample. The Erlenmeyer flask was then closed, and the sample was allowed to change color for 1 h. The samples were measured using a spectrophotometer at a wavelength of 640 nm.

Bacterial embedding was performed by dissolving alginate in a 4% (w/v) solution at 70 °C for 15 minutes, then allowing the temperature to decrease to 50 °C before adding the bacterial isolates. The bacterial isolates used in the anodes were *Pediococcus acidilactici*, while *Staphylococcus warneri* was used in the cathodes. The electrodes to be coated were dipped in the alginate solution. Electrode plates were then inoculated with the bacterial isolates, and microbial density was assessed using a total microbial count. The total number of microbes in the biofilm layer on the electrodes was determined by swabbing the electrode surfaces and placing the swabs into tubes containing 10 mL of KH_2PO_4 buffer solution, followed by homogenization using a vortex to achieve a 10-fold dilution. A 10^1 dilution was prepared by adding 1 mL of the initial solution to 9 mL of physiological saline, with subsequent dilutions of 10^2, 10^3, and so Bacterial embedding was performed by dissolving alginate in a 4% (w/v) solution of 70^{o}C for 15 min, then waiting for the temperature to drop to 50 °C and adding bacterial isolates.

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$$N = \frac{C}{[(1 \times n1) + (0.1 \times n2)] \times d} \quad (1)$$

Description:

N	= Total colonies per mL or gram sample
C	= Number of colonies of all bowls within the calculation limit
n1	= Number of bowls at the first dilution
n2	= Number of cups at the second dilution
d	= First dilution level when starting calculation

Microbial fuel cell (MFC) design used in this study was a single-chamber MFC with one design. The vessel used was made of glass and measured 10×7×10 cm. Copper is used as the anode, and carbon garfit is used as the cathode. The cathode was attached to the vessel, and the anode was placed inside the vessel. The design of the MFC vessel is shown in Figure 1.

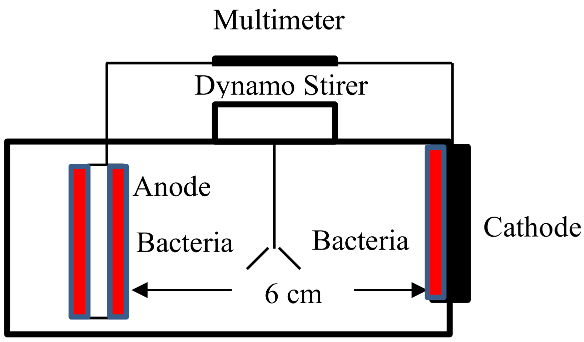

Figure 1. MFC single chamber design

MFC transformation was conducted by mixing fish wastewater with activated sludge in vessels containing electrodes colonized by bacteria. A total volume of 450 ml was used, with wastewater and activated sludge mixed at ratios of 10:1 and 11:0, respectively. Electric current was measured across four different treatments: electrodes (anode and cathode) without bacterial attachment, both with and without added activated sludge (KoL and Ko), and electrodes with bacterial isolates attached, both with and without activated sludge (AKL and AK). Electrical measurements of the MFC were taken hourly over a 120-hour period using a multimeter. Voltage and current values were recorded three times for each of the four treatments.

Results and Discussion

MFC Electrity of Fishery Wastewater

The electrical values measured in the MFC system for 120 hours were voltage (Figure 2), electric current (Figure 3), and electrical power (Figure 4). The voltage and electric current values were measured using a digital multimeter in units of volts (V) and milliamperes (mA), respectively. The power value is obtained by multiplying the voltage with the electric current, which is in milliwatt (mW) units.

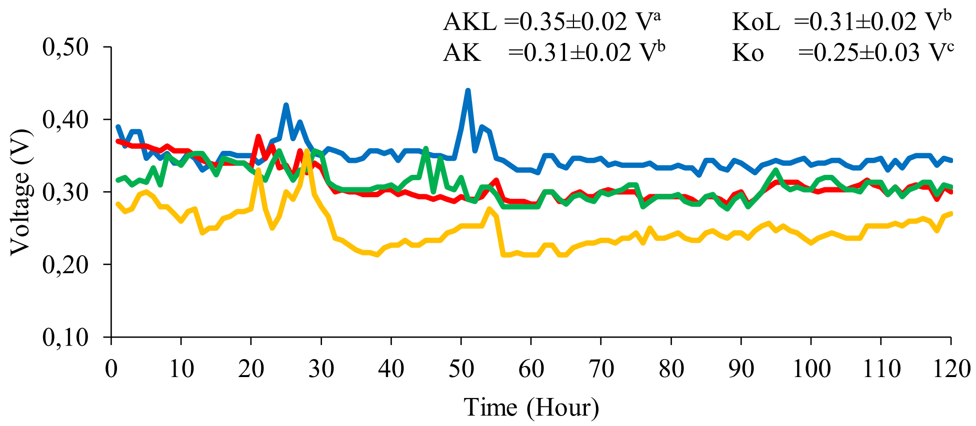

Figure 2. MFC voltage values

The voltage values obtained in this study fluctuated. ANOVA statistical test results ($p < 0.05$) indicated that the AKL treatment differed significantly from other treatments in the average electrical voltage produced by the MFC. The treatment involving bacterial attachment to the electrodes and the addition of sludge had a significant impact on the voltage generated by the MFC system. The addition of active sludge can increase the electrical output of the MFC because microbes convert the substrate into simpler forms and produce free electrons [2]. Bacterial adhesion to both electrode plates can generate higher electricity due to the close proximity of the bacteria to the electrodes, facilitating faster electron transfer [6].

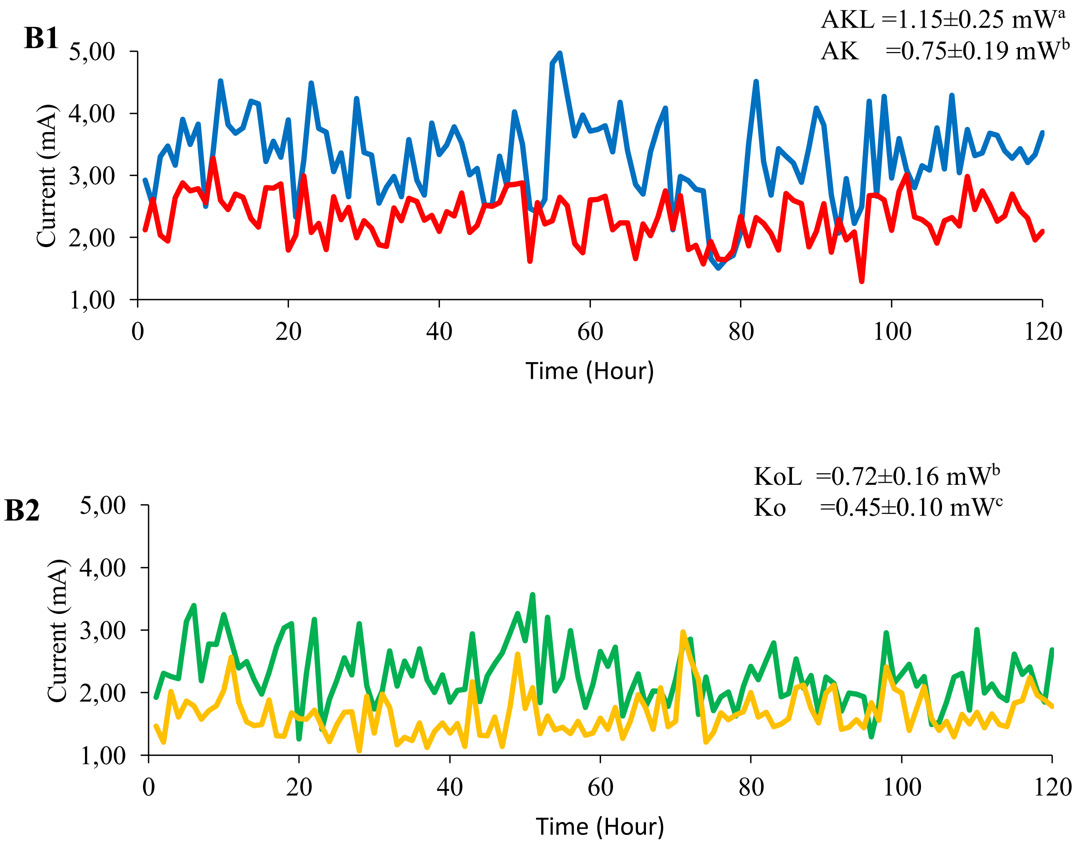

Figure 3. MFC electric current values

The electric current measured in this study fluctuated. Results from the ANOVA statistical test ($p < 0.05$) indicated that the average electric current was significantly influenced by the treatment involving bacterial attachment to the electrodes and the addition of active sludge. The AKL treatment exhibited the highest average electric current compared to the other treatments. Bacterial attachment on both electrode plates can generate a higher electric current because the bacteria reduce the resistance to electron transfer between the bacteria and the electrodes [6]. Lower resistance is a key factor in increasing the electrical output of microbial fuel cells (MFCs). In power generation systems, reduced resistance leads to higher electric current values.

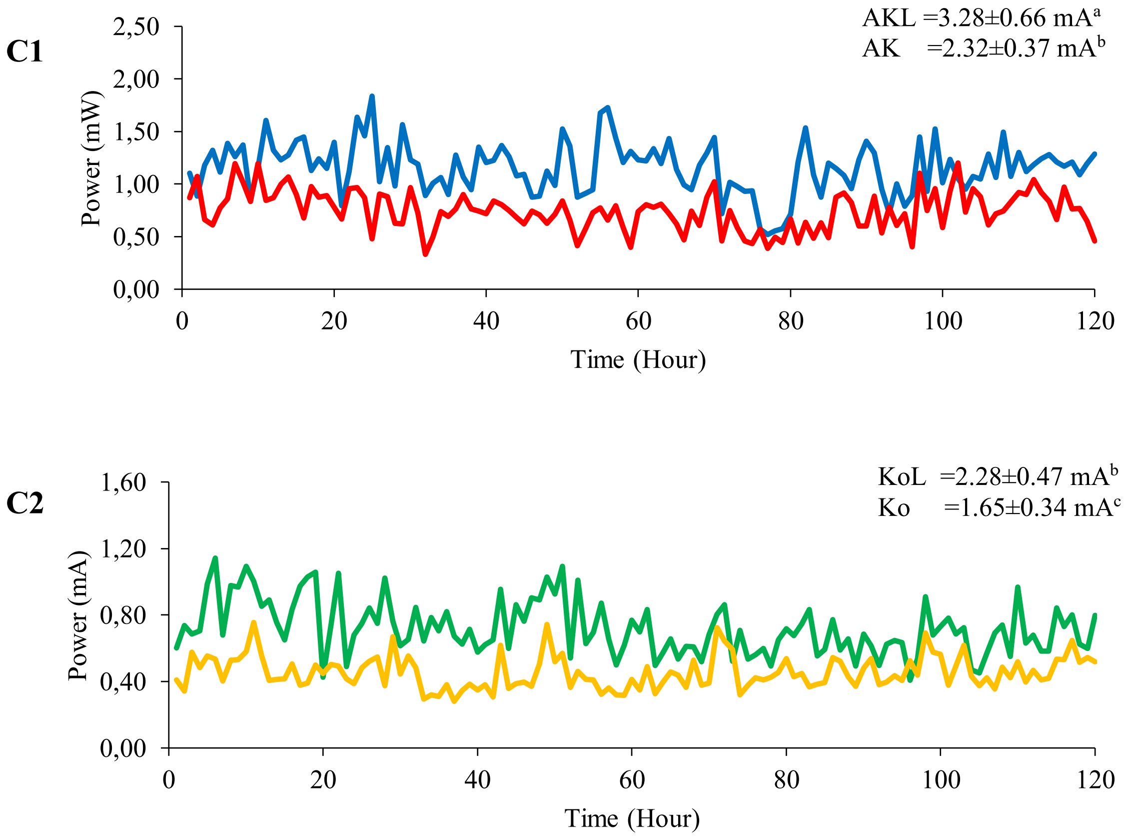

Figure 4. MFC electrical power values

The average electrical power generated by the MFC system, based on ANOVA test results ($p < 0.05$), was significantly influenced by the treatment involving active sludge addition and bacterial attachment on the electrode plates. The highest average electrical power value was observed in the AKL treatment, at 1.15 ± 0.25 mW, while the lowest was recorded in the Ko treatment, at 0.45 ± 0.10 mW. The higher electrical power in the AKL treatment compared to the other treatments may be attributed to the adherence of bacteria on the anode and cathode plates. Bacterial attachment to anodes and cathodes enhances the transfer of free electrons produced by bacteria to the electrodes in MFCs [6].

Performance of Wastewater Pollutant Load Reduction in MFC System

Wastewater characteristics are essential for determining changes in pollutant loads within MFC reactors. A reduction in pollutant load in the wastewater treated by the MFC system indicates the system's effectiveness [3]. Fishery wastewater contains a high concentration of nitrogen compounds and is typically evaluated based on several quality parameters, including Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Ammonia Nitrogen (TAN), and pH. The characteristics of fish processing wastewater are summarized in Table 1.

Table 1 Boiled fish wastewater quality changes during MFC process

Parameters	Boiled fish wastewater	Ko	KoL	AK	AKL	Quality standard of fishery wastewater *
pH	5.9 ± 0.1	6.4 ± 0.058^{a}	6.3 ± 0.058^{ab}	6.2 ± 0.058^{b}	6.2 ± 0.115^{ab}	6-9
COD (mg/L)	3540 ± 17.35	2273.96 ± 314.5^{a}	1700.16 ± 331.80^{a}	2146.45 ± 48.69^{ab}	1498.27 ± 159.39^{b}	150
BOD (mg/L)	345 ± 5	236.67 ± 2.89^{a}	226.67 ± 2.89^{b}	223.33 ± 2.89^{b}	213.33 ± 2.89^{c}	75
TAN (mg/L)	0.62 ± 0.06	0.44 ± 0.13^{a}	0.44 ± 0.09^{a}	0.55 ± 0.03^{a}	0.05 ± 0.03^{b}	5

Source : [PERMEN-LH] Kementrian Lingkungan Hidup (2014)[18]

Value of COD

The results showed that the COD values decreased differently across each treatment. The largest reduction in COD was observed in the AKL treatment, with a 57% decrease and a post-treatment COD value of 1498.27 ± 159.4 mg/L. ANOVA statistical test results ($p < 0.05$) indicated that the active sludge treatment had a significant effect on COD reduction. Elevated COD levels in wastewater can result from incomplete degradation of organic substances. Organic matter in wastewater can be fully degraded within 20 days [7].

The activated sludge added to the MFC system aimed to increase the number of microorganisms capable of accelerating the degradation of organic substances present in wastewater [8]. Wastewater treatment using MFC technology, in which electrodes are attached to bacteria, resulted in a 31.28% reduction in COD levels [9]. This decrease in COD occurs because organic compounds are continuously broken down by microbial activity at the anodes, which decompose the organic matter in the wastewater [10].

Value of BOD

The results showed a decrease in BOD values across all treatments. The greatest reduction was observed in the AKL treatment, with a 38% decrease and a post-treatment MFC value of 213.33 ± 28.9 mg/L. ANOVA statistical analysis ($p < 0.05$) indicated that both activated sludge treatment and bacterial paste treatment had a significant effect on reducing BOD levels. The decrease in BOD values can be influenced by several factors, including microbial metabolic rate, particle density, and environmental conditions [11]. The reduction of BOD in fishery wastewater is primarily caused by the degradation of organic substances through bacterial activity. Bacteria in activated sludge grow and develop, decomposing organic components by utilizing them as a food source [12].

Value of TAN

The results showed a decrease in TAN values that varied with each treatment. The most significant decrease in TAN was observed in the AKL treatment, with a 92% decrease and a post-treatment MFC value of 0.05 ± 0.03 mg/L. ANOVA statistical test results ($p < 0.05$) indicated that both activated sludge treatment and bacterial paste treatment had a significant effect on the reduction of TAN values.

The decrease in TAN value is influenced by nitrite and nitrate compounds formed through the ammonia oxidation process [13]. This oxidation generally involves nitrifying bacteria that use oxygen during the process. As a result, the oxidation of ammonia causes the TAN content to decrease and leads to an increase in the number of bacteria.

Value of pH

The results showed an increase in pH values that varied with each treatment. The pH increase in this study ranged from 5% to 8%. The largest increase was observed in the Ko treatment, with an 8% rise and a post-MFC value of 6.4 ± 0.06. ANOVA statistical test results ($p < 0.05$) indicated that bacterial paste treatment has a significant effect on the in pH value. The pH measurements demonstrated an increase across all treatments. This rise in pH is attributed to the presence of organic compounds such as trimethylamine, which result from the activity of organic matter and microorganisms [14]. Additionally, the increase in pH may be caused by a decrease in CO_2 levels due to oxygen consumption during the decomposition of organic substances.

Microbial Density in Biofilms on Electrode Plates

The electricity generated in the Microbial Fuel Cell (MFC) system is influenced by several factors, one of which is the density of microbes. Microorganisms play a crucial role in degrading the organic substances contained within the MFC system. These microorganisms utilize organic compounds such as fats, carbohydrates, and proteins as nutrient sources to produce energy [15]. The calculated microbial densities on the anode and cathode plates are presented in Table 2.

Table 2 Microbes density on anode and cathode

Parameter	Electrodes	Electrode plates initial attachement	After transformation process in MFC			
			Ko	KoL	AK	AKL
TPC	Anode	1.03×10^3	1.43×10^1	4.07×10^6	3.64×10^6	2.86×10^6
(cfu/cm^2)	Cathode	4.29×10^6	4×10^3	3.16×10^8	1.70×10^8	4.43×10^8
TPC	Anode	3.01	1.13^b	6.60^a	6.56^a	6.45^a
(Log)	Cathoda	6.72	3.6^c	8.49^a	8.21^b	8.64^a

The results were analyzed using statistical tests by converting the TPC values into logarithmic values. ANOVA statistical test results ($p < 0.05$) showed that the treatment involving the addition of active sludge and bacterial paste on the electrode plate had a significant effect on the microbial density on the anode plate. Similarly, ANOVA results ($p < 0.05$) indicated that the application of bacterial paste on the electrode plate and the addition of active sludge significantly influenced the microbial population on the cathode plate.

Differences in microbial densities between anode and cathode biofilms can result from variations in oxygen and nutrient availability during the MFC process. Biofilms on cathodes, which are colonized by bacteria, reach their maximum microbial growth more rapidly than biofilms on anodes. This accelerated bacterial growth in cathode biofilms is attributed to the rate of oxygen consumption in the cathode, which is approximately twice as fast as that in the anode [16]. Microbial density within the MFC system influences the electrical output; a moderate bacterial density allows protons generated from bacterial metabolism to efficiently reach the electrodes, thereby producing a higher electrical output [17].

Conclusions

The interaction between activated sludge and bacterial fixation on the electrode plate has a significant influence on the electrical output of the microbial fuel cell (MFC), the pollutant load in fish-processing wastewater, and the microbial density on the electrode surface. Embedding bacteria and adding activated sludge enhance the electricity generated by the MFC, with the AKL treatment showing the best performance. For future improvement, optimizing the ratio of sludge to bacterial inoculum, modifying the electrode surface to promote biofilm formation, and exploring alternative electrode materials with higher conductivity could further improve MFC efficiency. Additionally, integrating real-time monitoring of microbial activity and pollutant degradation could provide deeper insight into system stability and long-term performance.

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